

Slawghterhouse waste recycling improve production and nutritional quality of black soldier fly larvae (*Hermetia illucens*)

ABSTRACT

The objective of the study was to assess the effect of the composition of various organic substrates on the productivity and nutritional quality of black soldier fly (BSF) larvae. Four treatments (T1, T2, T3, T4) are based on organic materials (brewer's grains, palm kernel cake, soybean okara, (*Moringa oleifera*) leaves, marine fish and poultry viscera) in defined proportions. The experimental set-up used is a completely randomized block of 12 tanks arranged in triplicate. The loading density was 5750 larvae per kg of substrate. The experiment lasted 14 days. No significant difference was obtained between the pH values ($p>0.05$) of treatments T1, T3 and T4. The highest results for moisture, total nitrogen, and assimilable phosphorus were obtained in treatments T3 and T4 ($p<0.05$). Also, the best (final weight, weight gain per larva and survival rate) and nutritional qualities (proteins and lipids) were recorded in BSF produced from treatments T3 and T4 ($p<0.05$). We can therefore conclude that the quality of the production substrate influences the productivity and quality of the BSF produced. A substrate rich in chemical and nutritive elements (slawghterhouse waste) improve productivity and quality of BSF. Its use is recommended for sustainable raising of animal and aquaculture species.

Keywords: slawghterhouse waste, organic substrates, black soldier fly larvae, productivity and quality, sustainable raising.

1. INTRODUCTION

One of the challenges that the development of fish farming faces is fish food. Fish food represents the highest proportion of the total cost of fish farming production. It represents 78% of the cost of tilapia (*Oreochromis niloticus*) production in ponds, 35% for tilapia production in ponds and 62% of total (*Clarias gariepinus*) production costs [1]. Consequently, good food management is a

determining factor, to a very large extent, in the viability and profitability of fish farming businesses.

However, most of the ingredients used to formulate protein foods for fish farming, such as fishmeal and soya meals, are primarily intended for human consumption [2]. It is therefore becoming imperative to find new sources of non-conventional proteins that are ecologically sustainable and locally accessible to meet the future needs of the animal industry. The black soldier fly (BSF) has proved its worth and can be used for this purpose [2]. Several studies carried out on this species report nutritional properties comparable to fishmeal and soybean meal traditionally used in farm animal rations [3,4,5]. The BSF (*Hermetia illucens*) are highly prolific and can easily be raised locally. It is often raised on substrates consisting of agricultural co-products or residues from agri-food processing [6,7]. Various substrates are used to produce BSF larvae, but the productivity varies despite the production conditions. [8,9] showed that *Hermetia illucens* larvae performances varied with the substrate used. Consequently, the raising of *Hermetia illucens* requires the knowledge of the quality of the raw materials available to make up its living substrate.

The composition of BSF larvae such as fatty acid profile can be influenced by the type of food waste or organic matter. The concentration of C16:1 was the highest in the tofu by-product treatment. The BSF larvae produced by tofu by-products show benefits for larval growth and nutrient accumulation [10]. Its content of oleic acid and linolenic acid was higher compared with other treatments. According to [11], substrate composition influences the nutritional value of larval BSF. Diversity of the BSF larvae substrate production has been identified by [12]. Agriculture through products/ingredients with a very good profile in essential amino acids and polyunsaturated fatty acids [13,14,15] were identified and incorporated into larval BSF production substrates in the present experiment.

The present study aimed to assess the effect of the quality of various organic substrates on the productivity and nutritional quality of BSF larvae.

2. MATERIALS AND METHODS

2.1. Study area and origin of the pupae

The experiment was carried out at the Center Pilote of Production Agriculture (CePiPa) in the village of Ko-Anagodo, district of Tchaada in the township of Ifangni. Six kg of BSF fly pupae were used.

2.2. Experimental Design

The experimental setup used was a completely randomized block design. Six raw materials were used to make up the production substrate, namely brewer's grains, palm kernel cake, soya okara, *Moringa oleifera* leaves, marine fish and poultry viscera all used in their fresh state.

2.3. Conduct of the experiment

Black soldier fly larvae were sown at a density of 5750 larvae/kg of substrates. Four treatments (T1, T2, T3 and T4) were tested in 12 tanks of 25 liters randomly distributed. 3 repetitions for each treatment were used. The growth substrates used for the test were made up of available raw materials. Each tank contained 4 kg of substrate which composition of substrates per treatment is presented in Table 1.

Table 1: Composition of experimental substrates

Ingredients (%)	T₁	T₂	T₃	T₄
Soya Okara	50	40	30	30
Palm kernel cake	25	25	25	25
Brewing grains	25	25	25	25
Poultry viscera	0	0	0	20
Marine fish viscera	0	0	20	0
<i>Moringa oleifera</i> leaves	0	10	0	0
Total	100	100	100	100

The physicochemical quality (temperature, pH, humidity) of the substrate was assessed twice a day. Measurements were taken twice a day in each tank during the 14 days of experimentation. Black soldier fly larvae were counted manually using a 2-millimeter mesh sieve to determine the initial and final average weights with electronic balance of precision of 0.1g.

2.4. Zootechnical parameters studied

At the end of this experiment, several zootechnical parameters were calculated to estimate the growth and productivity of black soldier fly larvae.

- ✓ Initial Average Weight (IAW)

IAW (g) = IB/IN where IB is the Initial Biomass (g), and IN is the Initial Number.

- ✓ Final Average Weight (FAW)

FAW (g) = FB/FN where FB is the Final Biomass (g), and FN is the Final Number.

- ✓ Weight Gain (WG)

WG (g) = FAW - IAW where IAW is the Initial Average Weight (g), FAW is the Final Average Weight (g)

- ✓ Efficiency Conversion food Ingest (ECI)

ECI (%) = FB / ISM x 100 where FB is Final Biomass (g) and ISM is the Initial Substrate Mass.

- ✓ Substrate Degradation Rate (SDR)

SDR (%) = (ISM - RSM) / ISM x 100 where ISM is the Initial Substrate Mass and RSM is the Remaining Substrate Mass.

- ✓ Daily Average Gain (DAG)

DAG (g/d) = (FB - IB) / D where IB is the Initial Biomass (g), FB is the Final Biomass (g), and D is the duration of the experiment (days).

- ✓ Survival Rate (SR) of larvae

SR (%) = (FN - IN) / IN x 100 where FN is the Final Number, and IN the Initial Number per tank.

2.5. Determining the nutrient composition of production substrates

The composition of total nitrogen, total phosphorus and potassium along with the dry matter and ash content were determined for the different substrates at the laboratory of soil sciences at Abomey-Calavi University using the following methods: pH (water) and pH (KCl) were determined respectively by the potentiometric method with a soil/distilled water ratio of 1/2.5 and a soil/KCl 1 N solution ratio of 1/2.5 [16]. Total nitrogen was determined to use the Kjeldahl method [17]. The content of assimilable phosphorus (P_{ass}) was determined to use the Bray 1 method [18]. Exchangeable potassium was determined to use the [19] method. The level of organic matter was determined to use the method of [20].

2.6. Statistical Analysis

Statistical analysis was performed using the STATVIEW program (version 5.01) at a probability level of 5%. Analysis of variance with a classification criterion (ANOVA 1) was used to check the normality and homogeneity of the data. Fisher's LSD test was also used to make paired comparisons of the different means.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. BSF larvae production substrates physico-chemical parameters

The physicochemical parameters (temperature, pH, moisture) of the BSF larvae substrate production were monitored throughout the experimental period to assess the raising conditions for the larvae. The averages and standard deviations of these parameters during our test are shown in Figures 1, 2 and 3.

pH values in evening are lower than that of the morning in treatments T2, T3 and T4 (figure 1).

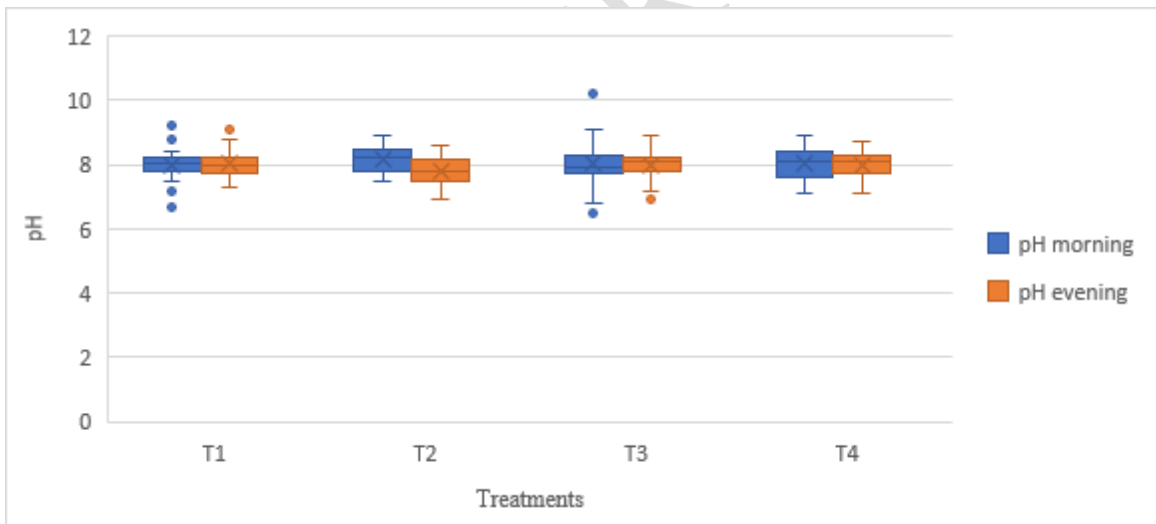


Figure 1. Variation of pH in different treatments during BSF larvae production

Figure 2 shows the evolution of moisture during the production of BSF larvae in the different substrates. The average level of moisture in the evening is slightly higher than that of the morning in all treatments.

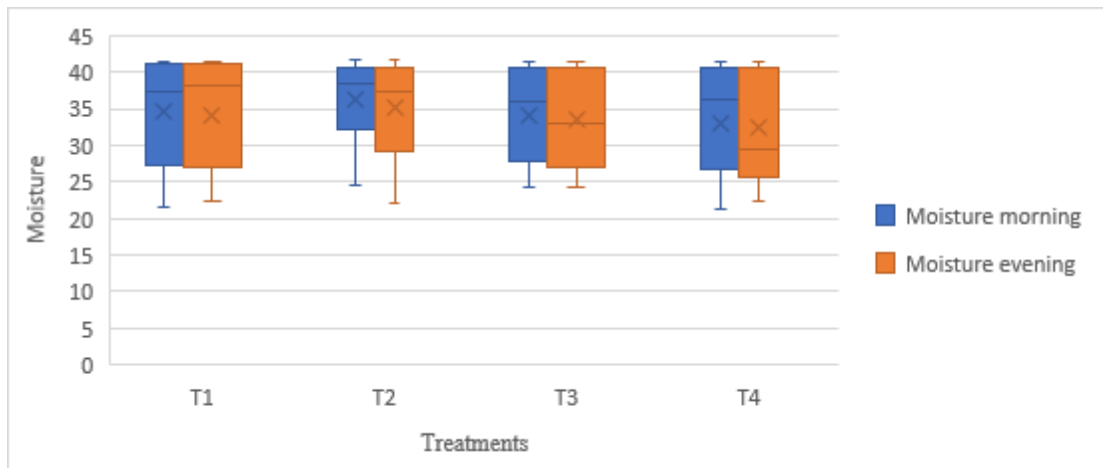


Figure 2. Variation of moisture in different treatments during the BSF larvae production

Figure 3 shows the variation in substrate temperature for the production of BSF larvae in the different treatments. The average temperature maintained the same trend in all treatments, with a slight increase in the evening as opposed to the morning.

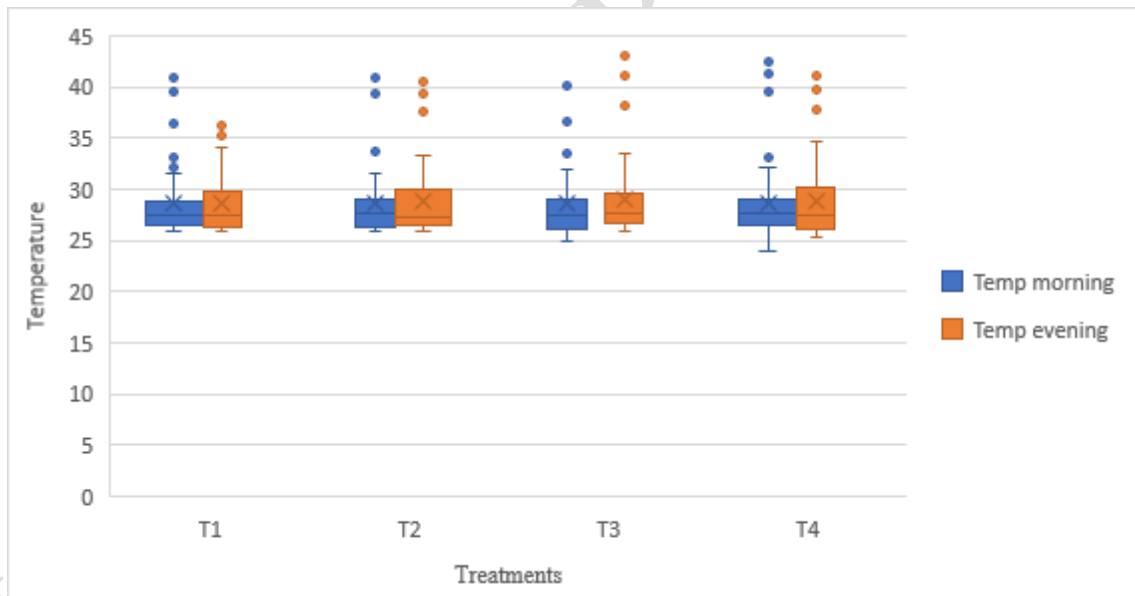


Figure 3. Variation of temperature in different treatments during BSF larvae production

3.1.2. Productivity of BSF larvae

The productivity of MSN larvae after 14 days of experimentation is presented in Table 2. These results showed that there is no significant difference ($p > 0.05$) between the treatments (T1, T2, T3 and T4) for final biomass (FB), final average weight (FAW), weight gain (WG), substrate

degradation rate (SDR) and survival rate (SR). Final average weight (FAW) and final biomass (FB) during the test varied respectively from 0.031 ± 0.01 g (T2) to 0.056 ± 0.02 g (T4) and 2046.92 ± 179.78 g (T2) to 3872.45 ± 493.92 g (T4). The survival rate varied from $94.46\pm 3.48\%$ (T2) to $99.17\pm 0.85\%$ (T4). The substrate degradation rate progressed from 13.37 ± 0.56 (T3) to $19.63\pm 4.96\%$ (T4). The daily average gain (DAG) recorded during the test varied significantly between T2 and T4 ($p<0.05$). Figure 5 shows that this variation is from 0.58 ± 12.84 g/d (T2) to 44.05 ± 35.27 g/d (T4). The best Efficiency Conversion food Ingest (ECI) was obtained with T3 and T4 ($p<0.05$). Overall, T3 and T4 are the best substrates for producing black soldier fly larvae.

Table 2: Black soldier fly larvae productivity parameters

Parameters	T1	T2	T3	T4
IB (g)	674.05 ± 0.00^a	674.05 ± 0.00^a	674.05 ± 0.00^a	674.05 ± 0.00^a
IMW (g)	0.03 ± 0.00^a	0.03 ± 0.00^a	0.03 ± 0.00^a	0.03 ± 0.00^a
FB (g)	2321.42 ± 19.69^b	2046.92 ± 37.99^a	2835.78 ± 8.89^a	3872.45 ± 0.722^a
FMW (g)	0.03 ± 0.01^a	0.03 ± 0.01^a	0.04 ± 0.01^{bc}	0.06 ± 0.02^c
WG (g) / larvae	0.005 ± 0.00^a	0.002 ± 0.00^a	0.013 ± 0.00^c	0.027 ± 0.00^{cd}
ECI (%)	19.34 ± 3.42^{cb}	17.05 ± 4.49^c	23.63 ± 6.70^b	32.27 ± 12.34^a
SR (%)	96.15 ± 2.56^a	94.46 ± 3.48^a	95.88 ± 1.77^a	99.17 ± 0.85^b
SDR (%)	80.34 ± 4.96^a	86.55 ± 2.08^a	86.63 ± 0.56^a	83.92 ± 1.66^a

Values are expressed as mean \pm standard deviation. Values in the same line with a letter in common are not significantly different ($p>0.05$).

3.1.3. Nutrient quality of black soldier fly larvae and production substrates

The biochemical composition of the substrates production of BSF larvae (table 3) varied significantly according to parameters in the treatments ($p<0.05$). pH values vary from 4.53 ± 0.05 (T2) to 4.75 ± 0.02 (T1). Moisture values varied from $60.57\pm 2.59\%$ (T1) to $65.11\pm 2.26\%$ (T3). There was a significant difference ($p<0.05$) between treatments T1 and T3. Nitrogen total values ranged from $1.70\pm 0.05\%$ (T3) to $2.66\pm 0.31\%$ (T4). There was no significant difference ($p>0.05$) between treatments for organic matter and ash content.

Table 3: Biochemical composition of the production substrates of BSF larvae

Treatments	T1	T2	T3	T4
pH	4.75±0.02 ^a	4.53±0.05 ^b	4.71±0.01 ^a	4.73±0.02 ^a
Moisture (%)	60.57±2.59 ^b	64.52±1.89 ^{ab}	65.11±2.26 ^a	64.73±2.01 ^{ab}
Nitrogen total (%)	1.88±0.19 ^b	1.87±0.27 ^b	1.70±0.05 ^b	2.66±0.31 ^a
Assimilable phosphorus (ppm)	16.85±0.76 ^a	17.47±0.47 ^a	16.72±0.36 ^a	16.30±1.01 ^a
Potassium (meq/100g)	0.39±0.06 ^{ab}	0.47±0.04 ^a	0.28±0.07 ^b	0.31±0.18 ^b
Dry matter (%)	39.43±3.23 ^a	35.48±2.37 ^b	34.89±2.69 ^b	35.27±1.96 ^b
Ash (%)	4.61±0.39 ^a	4.53±0.22 ^a	4.69±0.13 ^a	4.74±0.21 ^a
Organic matter (%)	95.39±0.41 ^a	95.47±0.35 ^a	95.31±0.57 ^a	95.26±0.13 ^a

Values are expressed as mean ± standard deviation. Values in the same line with a letter in common are not significantly different ($p>0.05$).

Table 4 presents the biochemical composition of BSF larvae produced from the substrates during our experiment. A significant difference ($p<0.05$) variation of biochemical composition parameters according to the treatments is observed ($p<0.05$). The best protein and ash values of BSF larvae are obtained with T3.

Table 4: Biochemical composition of black soldier fly larvae

Biochemical composition by treatment (%)	T1	T2	T3	T4
Protein	44.62±0.06 ^b	43.88±0.42 ^c	45.5±0.13 ^a	43.63±0.56 ^c
Fat	36.15±0.04 ^{ab}	35.95±0.04 ^c	36.21±0.02 ^a	36.09±0.01 ^b
Dry matter	68.37±0.31 ^b	61.46±0.42 ^c	67.49±1.03 ^b	72.6±1.09 ^a
Ash	13.30±0.08 ^c	13.75±0.17 ^b	14.78±0.10 ^a	10.33±0.24 ^d

Values are expressed as mean ± standard deviation. Values in the same line with a letter in common are not significantly different ($p>0.05$).

Figure 4 shows the influence of chemical parameters composition (moisture, organic matter content, pH, total nitrogen, assimilable phosphorus, dry matter, ash and potassium) of the substrate on zootechnical and nutritional performances of BSF larvae. In terms of zootechnical and nutritional performances, the decomposition rate of the substrates and the initial average weight (IAW) and final average weight (FAW) of the larvae are presented.

This figure shows that all the chemical and zootechnical parameters are found in the positive parts of axes 1 and 2 (in the 1.5 boundary definition zone and the negative bounds with no parameters). This observation proves that these parameters are not unfavorable for BSF larvae production. There is therefore a correlation between abiotic and biological factors of production. This means that humidity, organic matter content, pH, total nitrogen, assimilable phosphorus, dry matter, ash and potassium are all at reasonable values for a good rate of substrate decomposition, hence the reasonable average weights obtained. This implies that physicochemical parameters generally influence BSF larvae growth parameters and chemical composition. Specifically, the figure shows that total nitrogen (protein) levels are linked to pH and larval survival. All these parameters therefore influenced the level of productivity of each substrate obtained.

UNDER PEER REVIEW

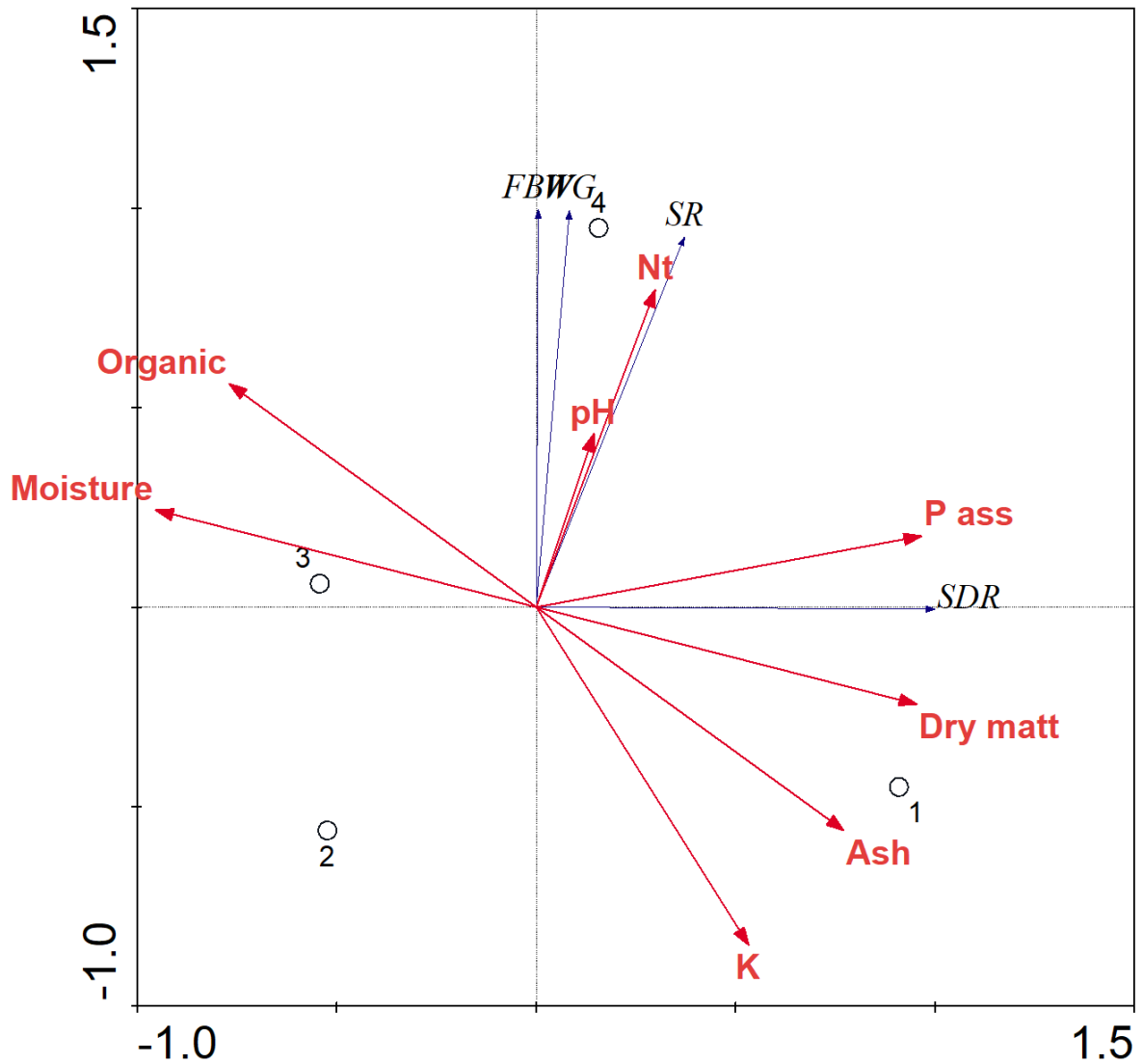


Figure 4: Influence of substrate chemical parameters on BSF larvae zootechnical and nutritional performances

Figure 5 shows the variation of Daily Average Gain (DAG) in different treatments. There is a significant difference ($p < 0.05$) according to the treatments. The best DAG are obtained with treatments T3 and T4.

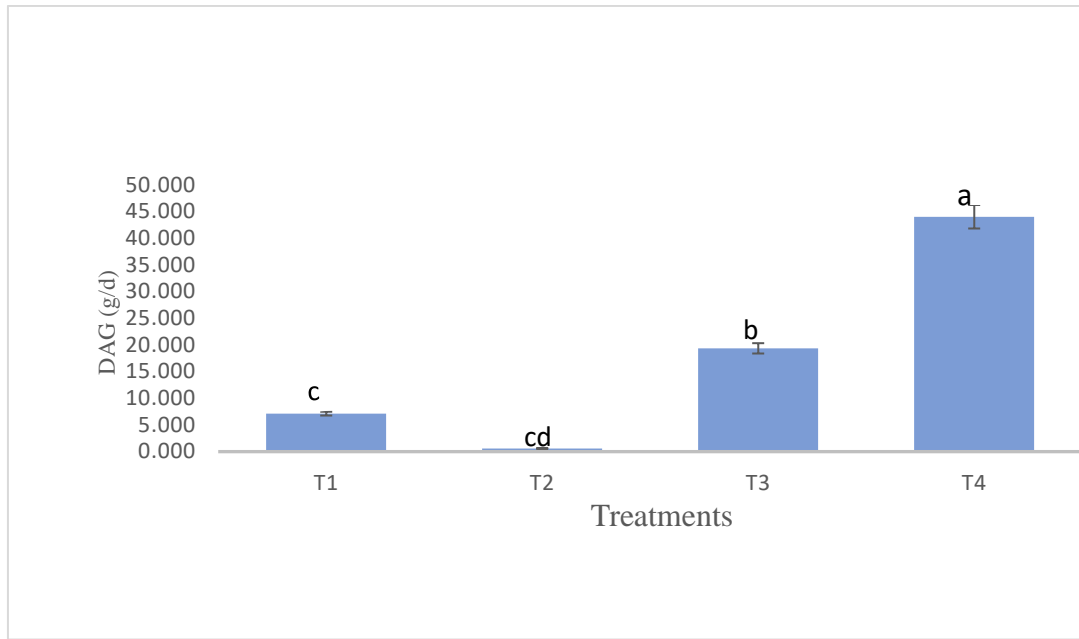


Figure 5. Variation of Daily Average Gain in different treatments during BSF larvae production

3.2. Discussion

Variations in physicochemical parameters in grow-out tanks are within the tolerances of *Hermetia illucens*. [21] reported that the ecological requirement of the BSF is between 24°C and 36°C. The variations in average temperature (28.60°C to 28.87°C) observed during this experiment are within this range. [22] demonstrated that BSF larvae have a better survival rate when the pH is between 6 and 10. These values coincide with those found in this study (7.97 to 8.02). [23] demonstrated that the more BSF larvae consume their food substrate, the more heat is released, due to intense microbial activity, and the higher the substrate temperature is. This may justify the maximum temperature values obtained at the start of the experiment in the tanks. The drop in temperature in our trial is most likely attributable to the reduction in food intake as the larvae enter pupation [23,24].

The final average biomass weight ranged from 31±0.01 mg (T2) to 56±0.02 mg (T4). These values corroborate those found by [22] after mixing wheat bran and maize meal with the BSF larval grow-out substrate by varying the pH of the substrates. [22] showed in their study on the effect of the initial substrate pH on the biological growth of BSF larvae that the best survival rates (94.46±3.48% to 99.17±0.85%) were observed when the pH was between 6 and 10. This study

confirms the substrate pH and larval survival rates observed during this experiment. Also, [25] working on the effects of food waste moisture content on residue separation, growth and larval survival in BSF bioconversion found similar results. [26] assessed the effects of fecal sludge moisture content in BSF bioconversion at 65%, 75% and 85% moisture content, and reported that moisture content has a significant effect on the dry weight of foreskins, with the heaviest foreskins obtained at 85% moisture content. This hypothesis confirms the better results obtained for T3 and T4, which had the highest moisture content and best daily average gain (DAG) and Efficiency Conversion food Ingest (ECI) respectively.

Furthermore, analysis of the nutrient composition of production substrates indicates that the type of substrate used in production systems could have a significant influence on the productivity of BSF larvae. Raising black soldier flies requires the knowledge of the raw materials available to make up their living substrate. These BSF larvae can degrade organic waste compared with other species of raising insects [27,28]. They can reduce the total mass of organic household waste by 65.5 to 78.9% [29]. The total nitrogen content recorded in the substrates varied from 1.70% (T1) to 2.66% (T4). [30] reviewed the studies analyzing the conversion of organic resources by *Hermetia illucens* and found a higher conversion rate in waste containing more protein. This assertion is confirmed by the results of our studies where the T4 treatment containing more protein induced the highest productivity of BSF larvae. In addition, these results could also be due to the better physicochemical conditions (humidity, temperature, pH) in the T4 treatment compared with the other treatments. [31] reported that BSF larvae tolerate substrate humidity of between 30 and 90%.

According to [32], the best substrate reduction is observed on rich substrates in plant material compared to other substrate sources. This assertion is contrary to our results, since the rate of reduction or degradation did not vary significantly according to the treatment, even though animal co-products (fish or poultry viscera) were present in T3 and T4. This difference could be explained much more by the nutritional quality of the substrates. Indeed, [33] demonstrated that BSF larvae develop well on protein-rich substrates, particularly when a good nitrogen/carbohydrate balance is achieved. The carbohydrate material used here is brewer's grains, which are incorporated at the same rate in all treatments. Furthermore, [34] reported that in the recovery of organic waste, substrate reduction values (> 50%) also depend on the BSF larvae production technology.

The substrates also showed high levels of phosphorus (P) and potassium (K). The assimilable phosphorus content of the substrates varied from 16.30 ppm (T4) to 17.47 ppm (T2) and the potassium content varied from 0.28 meq/100g (T3) to 0.47 meq/100g (T2). According to [35], these residual substrates rich in N, P and K make excellent organic fertilizers for agriculture.

Nutritionally, the biochemical composition of BSF larvae in terms of protein content depended on the type of substrate as well as the stage of larval development [36]. In accordance with the results of the present study, it appears that the protein content varies depending on the substrates used. The protein content of BSF larvae from the different substrates varies from 43.63% to 45.5% respectively at the T4 and T3 treatments. These values are lower than those obtained (59.65% and 47.89%) by [37] and [12] respectively. Nevertheless, our results are slightly higher than that of 41.9% obtained by [38]. BSF larvae protein content can be estimated at approximately 42.1 [39]. The high protein level obtained at all the treatments could be explained by the use of soya okara in substrate composition. The BSF larvae produced by tofu by-products show an excellent protein content [10]. The lipid content of BSF larvae at the end of our study varied between $36.15 \pm 0.04\%$ to $36.21 \pm 0.02\%$. These values are lower than those (37.03 ± 0.81) found by [10]. This variation in the protein and lipid composition of BSF larvae can also be due to the technological diagram production used [40] and [41].

4. CONCLUSION

This study showed that biochemical composition of the substrate production had a major influence on larval productivity, such as the total larval yield and the individual body weight of the larvae. Protein content, humidity, temperature and pH of the substrate are determining factors for BSF larvae production in the level of yield. Substrates composed of brewers' grains, palm kernel cake, soya okara, poultry or marine fish viscera and crushed leaves of *Moringa oleifera* mixed in different proportions can be valued in BSF larvae production. The best productivity and quality of BSF larvae are obtained with treatments T3 and T4 composed of brewer's spent grain, soya okara, palm kernel cake and poultry or marine fish viscera. Also, using these substrates, allow a reduction in the maturity period of the BSF larvae and improve its quality as livestock food ingredients.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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